REMARKS

This application has been amended in a manner that is believed to place the application in condition for allowance.

Claims 1 to 4, 6 to 11, 14 and 16 have been amended to further clarify the present invention. Claims 28 to 40 have been added.

Claim 1 has been amended to recite a method that increases the synthesis of RNA. This is supported at least on page 4, lines 19-20 ("*increasing RNA synthesis*" or page 5, lines 1-3 "*methods (…) that significantly increase the yield of any RNA*").

In the same claim, step b) has been added to specify that a DNA template is added to a cell free system. This is supported at least on page 35, lines 9-12 of the specification: "*DNA template (…) were added to 25 µl of a premix (…) and E. coli S30 cell-free extract*".

Finally, the increase of the α subunit has been clarified by inserting the wording of the second paragraph of page 11 " … *so that the ratio of α subunit to other subunits concentration is increased*" in combination with the conventional subunit ratio that is disclosed on page 4, lines 22-24 "*precise stoichiometric ratio of two α, one β, one β' and one σ subunits*".

Claim 9 has been amended to specify the features of the DNA template, that now comprises a strong bacterial promoter with at least one UP element. The support is found at least on page 7,

lines 9-11: "*the DNA template comprises a strong bacterial promoter with at least one UP element*".

The increase of the production of protein by the method is supported at least on page 4, lines 19-20 ("*increasing protein synthesis*" or page 5, lines 1-3 "*methods (…) that significantly increase the yield of any protein production*").

Moreover, the optional behavior of step c (adding a thermostable polymerase) is now the object of new claim 28.

New claims 28 to 39 have been added and are supported as follows:

Claim 28 is the previous step c) of claim 9.

Claim 29 is the previously preferred embodiment of claim 3.

Claims 30 and 31 indicate the nature of the DNA template and are supported at least respectively on page 7, lines 11-12 and page 7, lines 16-18.

Claims 32 and 33 are the previously preferred embodiments of claim 14.

Claims 34 and 35 relate to the heterologous nature of the α subunit and found support at least respectively on page 6, lines 4-5 and on page 6, lines 10-11.

Claim 36 defines the UP element as disclosed on page 7 lines 6-7.

Claims 37 and 38 describe the bacterial extract origin, as mentioned on page 12, lines 16-17 and page 12, lines 18-20 in

relation with Table 1 on page 27.

Claim 39 relates to the preparation of the α subunit under a purified form, and is supported at least on page 11, lines 20-25.

Claim 40 is a combination of previous claims 1, 2, 6 and 8.

The specification has been objected to for missing numerous letters at the top or bottom of several pages, and for missing the text on the left side of Table 1. After reviewing the application, applicants believe that the specification as filed actually contained the allegedly missing letters and left sided table. Rather, applicants believe that the missing portions of the text must have occurred during the scanning and processing of the application. As a result, applicants submit herewith another copy of the specification as filed and request that this objection be withdrawn.

In the outstanding Official Action, claims 1, 8, 9 and 14 were objected to. Claims 1 and 9 now contain the word "*and*" before the last steps of the claimed methods. Claim 8 has been amended to recite "*the cell free extract*". Claim 14 has been amended to add the term "*of*" after downstream.

Withdrawal of these claim objections is therefore respectfully requested.

Claims 1-8, 11 and 14 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to

particularly point out and distinctly claim the subject matter which applicant regards as the invention. This rejection has been obviated by the amendment of these claims.

More specifically, claim 1 has been amended to indicate that the DNA template is added to the cell free system and the previously unclear expression "enabling RNA or polypeptide synthesis from a DNA template" has been deleted. Claim 1 has also been amended to clarify the expression "comparing to its natural concentration existing in the cell free system" to rather indicate that the normal stoichiometry (two α, one β, one β' and one σ subunits) is modified by adding α subunit, resulting in the increase of the ratio α subunit of RNA polymerase / other subunits.

Claim 3 has been amended and the content previously found after the term "*preferably*" is now the object of dependent claim 29.

Claims 7 and 11 have been amended by deleting the word "*comprised*", indicating that the concentration is ranging from 15 µg/ml to 200 µg/ml.

Claim 8 has been amended to refer to claim 2 instead of claim 6. A new claim 39 has been added to indicate that a subunit may also be added after purification of cells overexpressing it.

Claim 14 has been amended and the content previously found after the terms "*preferably*" and "*more preferably*" is now the object of respectively dependent claims 32 and 33.

Therefore, in view of the above, withdrawal of this rejection is respectfully requested.

Claim 1 was rejected under 35 U.S.C. §102(b), as allegedly being anticipated by Bowrin et al. (1994; FEMS Microbiology Letters, 15:1-6). For the following reasons, this rejection is respectfully traversed.

Claim 1 is directed to a method to increase RNA or polypeptide synthesis *i.e.*, increasing the yield of any RNA or protein in *in vitro* systems (page 5, lines 1-3), as compared to the same experiment without addition of α subunit. The increase in protein yield (thickness of the band) is apparent on Figure 6 comparing lane 1 (without added exogeneous factors, page 22, line 22) and lane 10 (3 µl of α subunit of *E. coli*; page 23, line 12 of the present specification).

Bowrin et al. discuss the production of a 140-bp lpp transcript (page 5) whose promoter is from *E. coli* (see Figure 1 from Nakamura et al. 1982; EMBO Journal, 1(6): 771-775). Figure 5 shows the results of the transcription of this 140-bp fragment in the presence (+) or absence (-) of ompR and/or subunit α.

Bowrin et al. conclude on page 5 (right column lines 1-7) that the cell-free production of the lpp transcript was not affected by the addition of the α subunit (compare the steady thickness of each band form lanes 1 to 4 of Figure 5). Bowrin et al. add that "*These results indicates that the inhibitory effect of α is clearly specific for the ompF transcript.*"

Therefore, Bowrin et al. do not disclose that addition of an α subunit increases the transcription, but rather that it has no inhibitory effect.

Consequently, Bowrin et al. do not disclose a method to increase RNA or protein synthesis from a template comprising a strong promoter and UP element, in a cell free system, by addition of the α subunit of RNA polymerase.

Therefore, in view of the above, withdrawal of this rejection is respectfully requested.

Claims 1, 2, 6, 7 and 9 to 14 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lesley et al. (1991; JBC, 266(4):2632-2638) in view of Bowrin et al. as evidenced by Bowrin, Brisette and Inouye (J. Bacteriol. 1992, 174(20): 6685-6687). For the following reasons, this rejection is respectfully traversed.

The present claimed invention discloses the unexpected observation that the addition of the α subunit alone (while the expression of other subunits remains unchanged; page 10, lines 11-12) leads to an increase in the yield of RNA or polypeptide (page 43, lines 19-22). This observation is surprising since before the present invention, only the effect of the addition of the entire RNA polymerase ($\alpha_2\beta\beta'\sigma$) or core polymerase ($\alpha_2\beta\beta'$) has been reported and shown to increase transcription. In this application, the inventors have reported that the addition of the α subunit alone (independently of other subunits) is sufficient

to drastically increase transcription (page 43 lines 19-22). Moreover, the combinations of the addition of the α subunit RNA polymerase with two other transcriptional elements, *i.e.*, the presence of a UP element in a strong promoter, and the elongation in the 3'UTR region, provide a great advantage to the cell-free RNA or protein synthesis.

Lesley et al. disclose the use of core RNA polymerase (2.4 µg was added to the reaction as disclosed on page 2633 paragraph bridging columns). Moreover, each upstream PCR fragment (used in the realization of the template DNA) contained a T7 RNA polymerase for transcription (page 2634, right column, second line).

Lesley et al. fail to disclose a method to increase RNA synthesis (claim 1), or polypeptide production (claim 9):

- first, from a template comprising a UP element as presently claimed. Rather, as mentioned above, all templates in Lesley et al. contained a promoter for a T7 RNA polymerase; and

- second, by adding specifically the α subunit of RNA polymerase. Rather, Lesley et al. report the addition of the core RNA polymerase, the core enzyme being defined as the entire RNA polymerase, as set forth in the present specification on page 3, lines 11 (said core enzyme $\alpha_2\beta\beta'$ ...).

Rather than being a method to improve synthesis of RNA or protein, the method disclosed in Lesley et al. is a rapid method to produce truncated proteins in small amounts (see page

2633, left column, lines 10-11). One possible application is the mapping of epitopes of mAbs. However, the method described in Lesley et al. does not resolve the problem of increasing the synthesis of RNA or polypeptide.

As mentioned above, Bowrin et al. do not disclose elements suggesting a method to increase RNA synthesis or a method to increase proteins production from a template comprising a strong bacterial promoter with at least one UP element by adding an α subunit.

Rather, Bowrin et al. report that the addition of an α subunit in a cell free extract may lead to contrary results, *i.e.*, an inhibition for some genes such as ompF, and an absence of inhibition for other lpp, but without giving any reason about these contrasting results. More interesting, is that Bowrin et al. do not show nor suggest that the α subunit may increase the transcription or the translation of a DNA template.

Applicants respectfully disagree with the observations of the Official Action that states that "*It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the cell free transcription-translation method and analysis of protein interactions of Lesley et al. to include the addition of plasmid pKI0033 and purified RNA polymerase alpha subunit and OmpR protein as taught by Bowrin et al. because Lesley et al. and Bowrin et al. teach it is within*

*the ordinary skill in the art to use in vitro transcription reactions to study protein interaction and transcription."*

Indeed, the person skilled in the art facing the problem of improving the synthesis of RNA or protein, would have, starting from Lesley et al., tried to modify the method, *i.e.*, to construct a DNA template with T7 RNA polymerase promoter and to add core RNA polymerase, rather than departing from this method. Lesley et al. do not disclose nor suggest first using different promoter elements or adding a particular subunit of the RNA polymerase, and even less the combination of these two features.

Even though the person skilled in the art may have considered both Bowrin et al. and Lesley et al., he or she would not have obtained the methods of the present invention since the method of Lesley et al. is based on using the T7 promoter and adding core polymerase to produce small amounts of proteins, whereas the method disclosed in Bowrin et al. give results that are dependent of the nature of the gene. Moreover, in absence of any explanation for the contrasting results obtained in Bowrin et al., it is not possible to anticipate the effect of α subunit overexpression, and even less to anticipate an increase in transcription, since Bowrin et al. never report such a phenomenon (only an absence of alteration; page 5, right column, lines 1-4).

A the time the invention was made, the person may have used *in vitro* transcription reactions, but certainly not to modify the method of Lesley et al. by replacing first the T7

promoter by a UP element and the addition of core polymerase by the sole α subunit. None of the prior art cited by the Official Action suggests using the particular UP element with a strong bacterial promoter in combination with the addition of an α subunit. The results shown in Bowrin et al. would have led the person skilled in the art away from using α subunit, since the results are dependent on the nature of the gene. The use of the lpp gene was a control and there is simply no disclosure concerning a particular element acting in the transcription process; neither in Bowrin et al. nor in Lesley et al. None of these two documents mentions the existence of an UP element with a strong bacterial promoter. Consequently, in view of the contrasting results of Bowrin et al. and in absence of any explanation, the person skilled in the art would lack a reasonable expectation of success of obtaining an increase of RNA and polypeptide, by adding an α subunit of RNA polymerase in a cell-free system, since the inhibition of transcription, is dependent from factors that are completely unknown.

Therefore, applicants submit that the present invention is not obvious in view of Bowrin et al. and Lesley et al.

Claims 1-3, 6, 7 and 9-14 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Lesley et al. in view of Bowrin et al. and Savchenko et al. (1998; Gene, 212:167-177). For the following reasons, this rejection is respectfully traversed.

As indicated above, Lesley et al. do not disclose nor suggest a method to increase synthesis of protein or RNA from a DNA template comprising a strong promoter and at least one UP element, by adding an isolated α subunit. Rather, this publication disclosed a method of producing small amounts of proteins based on T7 promoter and addition of a core polymerase.

As far as Bowrin et al. is concerned, it is directed to the overexpression of an α subunit, and its effect either on ompF (inhibition of transcription) or on housekeeping genes, such as lpp (no alteration in transcription). These contrasting effects have not been explained, nor particular elements identified.

Applicants submit that the person skilled in the art would not have departed from the method disclosed in Lesley et al., since this method is efficient for producing small amounts of proteins. The one of Bowrin et al. is dependent on the nature of the gene. Thus, in the absence of a reason for the contrasting results reported in Bowrin et al., it is not possible to anticipate the effect of α subunit overexpression, and even less to anticipate an increase in transcription, since Bowrin et al. never report such a phenomenon (only an absence of alteration).

Savchenko et al. mention that the arg promoter (Parg) contained a UP-like element at the -35 site of the promoter (page 175, right column, second paragraph). However, Savchenko et al.

is far from disclosing or suggesting a method to increase RNA or polypeptide synthesis, for the following reasons:

(1) The interaction between this UP-like element and the α subunit of the RNA polymerase has not been shown, only hypothesized. Therefore, the exact role of said UP-like element in transcription is pure speculation.

(2) This publication does not refer to a cell-free system, but rather to transformed cell cultures as explained on page 168, right column (2.1 bacterial strain and growth conditions), wherein it is indicated that *E. coli* cells were selected for their resistance to antibiotics (resistance conferred by the gene present on the plasmid used to transform cells).

(3) There is no mention of overexpression or addition of a particular transcription element in an *in vitro* system, particularly cell-free system, and even less the addition of α subunit. Indeed, in Savchenko et al. the experiments are carried out in *E. coli* cells, by a normal transcription system *i.e.*, the entire RNA polymerase present in these cells.

(4) Finally, Savchenko et al. suggest the presence of non-identified additional factors to explain the activation of this promoter (page 176, left column, first full paragraph).

The presence of a strong promoter is necessary but not sufficient to provide a method to increase RNA or polypeptide synthesis to a high level. As shown at least on page 44 of the present application, without addition of any factor, the efficiency of synthesis with a plasmid comprising PargC is 4.4% only (see Table 3, lane N°1, with explanation under the Table). In contrast, the addition of α subunit (lane N°10) results in a 13-fold increase (58.4%) and the addition of both α subunit and Tt RNP results in a 22-fold increase. As indicated on lines 14-16, the addition of α subunit to a strong promoter comprising at least one UP element unexpectedly leads in a cell-free system to the overexpression of the gene downstream of this promoter.

Consequently, Savchenko et al. by only disclosing a strong promoter coupled with the conflicting results of Bowrin et al. would not lead the skilled artisan to envisage modifying the method of Lesley et al., in order to obtain a method for increasing RNA or polypeptide synthesis in a cell free system.

Therefore, applicants submit that the present invention is not obvious in view of Bowrin et al., Lesley et al. and Savchenko et al.

Claims 1, 2, 6 and 7-14 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Lesley et al. in view of Bowrin et al. and Fujita et al. (1996; Methods in Enzymology, 273:121-130). For the following reasons this rejection is respectfully traversed.

As noted above, Bowrin et al. and Lesley et al. fail to disclose or suggest the claimed invention. The secondary reference to Fujita et al. disclose the reconstitution of RNA polymerase (holoenzyme) *i.e.*, the core enzyme (subunit composition $\alpha_2\beta\beta'$) and one $\sigma$ subunit (page 121, second and third lines). Moreover, Fujita et al. disclose the purification of an $\alpha$ subunit to obtain large amounts (5mg) and purity of more than 95% (page 123).

The application of these purified subunits is to reconstitute either a holoenzyme from a purified core enzyme with a two- to fourfold molar excess of $\sigma^{70}$ subunit (page 129, lines 3-5), or a holoenzyme by adding 6.6 nmol of $\alpha$, 3nmol of $\beta$ and 3 nmol of $\beta'$ to produce a holoenzyme having $\alpha_2\beta\beta'$ composition (typical reconstitution experiment, as indicated on page 129, lines 9-11). Fujita et al. fails to disclose or suggest overexpression of any of the subunits, except the $\sigma^{70}$ subunit. Nor does this publication disclose any reference to a UP element, to a method to increase RNA or polypeptide synthesis or to cell-free systems.

Consequently, Fujita et al. do not remedy the deficiencies of Lesley et al. and Bowrin et al.

Therefore, due to the above, the combination of references fails to render the presently claimed invention obvious.

Claims 1, 2, 4-7 and 9-14 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Lesley et al. in view of Bowrin et al. and Xue et al. (2000; Biochemistry, 39:14356-14362). For the following reasons, this rejection is respectfully traversed.

As noted above, Lesley et al. and Bowrin et al. fail to disclose or suggest the claimed invention.

The conclusions about the teaching of Bowrin et al. as well as Lesley et al. have been described above and applied in the same way for this rejection.

The secondary reference of Xue et al. disclose the purification of RNA polymerase from *Thermus thermophilus* and the characterization of its properties on a λPr promoter (page 14360, left column, second full paragraph). There is no mention in this publication of a gene expression study, neither in cells nor in a cell free-system, but rather of "abortive initiation assay" and "stalled elongation complex formation" on buffers comprising DNA and the RNA polymerase (RNAP) [page 14358, right column, (ii) and (iii)].

Xue et al. never mention nor suggest using a RNAP with a modified subunit ratio. Rather, the authors indicate on page 14356, right column, first full paragraph that *T. thermopilus* RNAP has a highly conserved composition ($\alpha_2\beta\beta'\sigma$), and it is the purified RNAP that they use in their experiments. There is simply no disclosure in Xue et al. of the use of a modified RNAP subunit

ratio, and even less the addition of an α subunit. Moreover, the experiments in Xue et al. were carried out with a λPr promoter that has never been reported as a strong promoter nor a promoter comprising an UP element.

Finally, experiments have shown that the addition of Tt RNP alone is not sufficient to note an increase in transcription or polypeptide synthesis as indicated on Table 5, lane N°1 (page 47) with a 2.9% efficiency of synthesis. The sole addition of *E. coli* α subunit gives a percentage of 33.2 (lane N°8). Surprisingly, the addition of both α subunit and Tt RNP has a cumulative effect, as compared to Tt RNP or α subunit alone (lanes N° 3 to 7), in RNA or protein synthesis carried out in a cell-free system.

Consequently, the person skilled in the art facing the technical problem of increasing RNA or polypeptide synthesis, would not have found in Xue et al. explicit elements or suggestion to overexpress α subunit in combination with the addition of the *T. thermopilus* RNAP, in cell free system, with a strong promoter comprising at least one UP element, starting from Bowrin et al (and contrasting results) and Lesley et al. (T7 promoter).

Therefore, applicants submit that the present invention is not obvious in view of Bowrin et al., Lesley et al. and Xue et al.

Claims 1, 2, 6, 7 and 9-16 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lesley et al. in view of Bowrin et al. and Uptain et al. For the following reasons, this rejection is respectfully traversed.

The conclusions about the teaching of Bowrin et al. as well as Lesley et al. have been described above and are applicable to this rejection.

The secondary reference of Uptain et al. discloses the comparison of the action of various terminators (Table 1) on the termination of *E. coli* RNA polymerase on ssDNA template. These experiments were carried out in buffer (TGK-$B_{500}$M4; page 13549, left column) where a purified holoenzyme (page 13548, right column) was added at 40 nM.

However, this publication does not teach or even suggest a method to increase RNA or polypeptide synthesis in a cell free system with the addition of an α subunit from a DNA template with a strong promoter comprising at least one UP element. Indeed, in Uptain et al. the RNA polymerase was added as a purified holoenzyme, and the addition of any RNA polymerase subunit, such as the α subunit was not reported. Moreover, there was no mention of any UP element in this publication, nor of a strong promoter. Finally, the experiments were carried out in buffer and there was no suggestion to apply this termination assay in a cell-free system.

In the present invention, the prolongation of PCR products by a terminator of at least 3bp provides an additional benefit to the cell free synthesis explained above, by improving new transcriptions initiations at the UP-carrying promoter on the same DNA via releasing the core RNA polymerase. Therefore, the combinations of the three transcriptional elements *i.e.*, (1) the UP element in a strong promoter, (2) the addition of an α subunit RNA polymerase and (3) the elongation in the 3'UTR region, provide an advantage to the cell-free RNA or protein synthesis.

Consequently, the combination of these three transcriptional elements unexpectedly shows that high yield of RNA or polypeptide can be obtained in a cell free system, what constitutes an improvement of the previously known methods, by considerably increasing the production of RNA or polypeptide. Uptain et al. do not teach or suggest to improve the production of RNA or polypeptide, and even less the combination of two or three of these transcriptional elements.

Therefore, Applicants submit that the present invention is not obvious in view of Bowrin et al., Lesley et al. and Uptain et al.

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In the present invention, the prolongation of PCR products by a terminator of at least 3bp provides an additional benefit to the cell free synthesis explained above, by improving new transcriptions initiations at the UP-carrying promoter on the same DNA via releasing the core RNA polymerase. Therefore, the combinations of the three transcriptional elements *i.e.*, (1) the UP element in a strong promoter, (2) the addition of an α subunit RNA polymerase and (3) the elongation in the 3'UTR region, provide an advantage to the cell-free RNA or protein synthesis.

Consequently, the combination of these three transcriptional elements unexpectedly shows that high yield of RNA or polypeptide can be obtained in a cell free system, what constitutes an improvement of the previously known methods, by considerably increasing the production of RNA or polypeptide. Uptain et al. do not teach or suggest to improve the production of RNA or polypeptide, and even less the combination of two or three of these transcriptional elements.

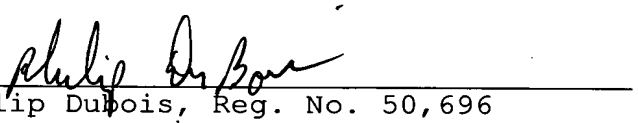
Therefore, Applicants submit that the present invention is not obvious in view of Bowrin et al., Lesley et al. and Uptain et al.

Please charge the fee of $50 for two extra dependent claims added herewith to Deposit Account No. 25-0120.

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

YOUNG & THOMPSON



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**APPENDIX**:

The Appendix includes the following item:

- copy of original specification as filed